

## From monoclonal antibody to gene for a neuron-specific glycoprotein in *Drosophila*

(neurodevelopment/retina/oligonucleotide probes/*in situ* chromosomal location)

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Contributed by Seymour Benzer, November 8, 1984

**ABSTRACT** A monoclonal antibody (MAb24B10), derived from mice immunized with *Drosophila* retina, exclusively stains photoreceptor cells in the retina and their axonal projections to the optic ganglia. The antigen (Ag24B10) is a 160-kDa glycoprotein comprising about 0.8% of the retina protein. By microsequencing, 19 of the first 21 amino acids at the NH<sub>2</sub>-terminal end of the protein have been determined. Using synthetic oligonucleotide probes corresponding to a portion of this amino acid sequence, we isolated a homologous  $\lambda$  genomic clone. A partial DNA sequence of this clone, along with blot experiments on genomic DNA and RNA, indicate that this clone is part of the structural gene for Ag24B10. By *in situ* hybridization, the gene was localized to the tip of chromosome 3R.

A remarkable chemical diversity of neurons is revealed by immunological and molecular biological studies (1–3). The mechanisms that generate this diversity, as well as the nature and function of neuron-specific molecules, are largely unknown. In the fruit fly, *Drosophila melanogaster*, one can gain clues to the function of a polypeptide by mutations affecting its structure. The basis of its developmental expression can be studied by using both classical and molecular genetic techniques. Our approach utilizes monoclonal antibodies (MAbs) to identify polypeptides associated with specific neuronal structures and to identify and study the genes encoding these antigens.

Among the various MAbs we have isolated that bind to different structures within the developing and mature retina, a subclass recognizes antigens that occur in photoreceptor cells (4, 5). These appear at different times during the maturation of these cells. We have described a membrane-associated glycoprotein identified by MAb24B10. It occurs only in photoreceptor cell bodies and their axons and appears at an early stage of maturation of these cells. An anatomical, developmental, and biochemical characterization of this antigen has been published (5). In this paper, we describe the isolation, partial characterization, and chromosomal mapping of the gene encoding this neuron-specific antigen.

### MATERIALS AND METHODS

**Synthesis and Labeling of Oligonucleotides.** Two 20-mer probe mixtures were kindly synthesized by S. Horvath in L. Hood's laboratory. Each mixture contained 32 different oligomers (probes I and II as indicated in Fig. 2). Approximately 0.1–0.2  $\mu$ mole of the 20-mer mixtures was purified by polyacrylamide gel electrophoresis. The 5'-OH ends were labeled using [ $\gamma$ -<sup>32</sup>P]ATP (7000 Ci/mmol; 1 Ci = 37 GBq) and T4 polynucleotide kinase (1.5 units) in 50 mM Tris, pH 7.6/10 mM MgCl<sub>2</sub>/5 mM dithiothreitol/0.1 mM EDTA. The <sup>32</sup>P-labeled oligonucleotides were purified by homochroma-

tography on DEAE-TLC plates, as described by Jay *et al.* (6). Their specific activity was 1–3  $\times 10^9$  cpm/ $\mu$ g.

**Probing the Genomic DNA Library.** A *Drosophila* genomic DNA library prepared by Maniatis *et al.* in  $\lambda$  Charon 4 (7) and provided by E. Meyerowitz was probed with the <sup>32</sup>P-labeled oligonucleotides. Plaques ( $\approx 3000$  per 15-mm Petri plate) were transferred to nitrocellulose filters, as described by Benton and Davis (8). The filters were washed in 50 mM Tris Cl, pH 8.0/1 M NaCl/1 mM EDTA/0.1% NaDodSO<sub>4</sub> for 1–2 hr at 42°C and then prehybridized for 1–2 hr at 42°C in 0.9 M NaCl/6 mM EDTA/0.09 M Tris Cl, pH 7.5/0.5% Nonidet P-40/5 $\times$  Denhardt's solution (0.1% Ficoll/0.1% polyvinylpyrrolidone/0.1% bovine serum albumin) containing yeast tRNA at 100  $\mu$ g/ml. The filters then were incubated with the <sup>32</sup>P-labeled oligonucleotides (1–2  $\times 10^8$  cpm) in prehybridization buffer plus 10% dextran sulfate at 52°C for 16–20 hr. The filters were washed in 6 $\times$  NaCl/Cit (0.09 M Na citrate, pH 7.0/0.9 M NaCl) for 3  $\times$  30 min at 4°C and then for 2 min at 52°C, dried, and exposed to Kodak XAR-5 film, with an intensifying screen, for 12–24 hr at –70°C.

**Preparation of DNA and RNA.** Genomic DNA was prepared from adult flies according to Meyerowitz *et al.* (9). Plasmid DNA was purified by the alkaline lysis method, as described by Maniatis *et al.* (10). DNA from the selected bacteriophage clone, designated  $\lambda$ Dm24B10, was also purified according to Maniatis *et al.* (10). DNA fragments for subcloning, DNA sequencing, and *in situ* hybridization were separated by electrophoresis in agarose or polyacrylamide gels, followed by electroelution in TBE buffer (45 mM Tris/45 mM boric acid/0.5 mM EDTA, pH 8.3), and then dialyzed against TEN buffer (10 mM Tris Cl, pH 7.6/1 mM EDTA/10 mM NaCl). The DNA fragments were extracted with phenol, concentrated with butanol, and precipitated with ethanol. *Drosophila* head and body RNA were prepared using a modification of the procedure described by Chirgwin *et al.* (11). Poly(A)<sup>+</sup> RNA was purified on oligo(dT)-cellulose, as described by Maniatis *et al.* (10).

**DNA and RNA Blots.** Restriction endonuclease digests of genomic DNA (1.7  $\mu$ g) and cloned DNA (0.6  $\mu$ g) were electrophoresed, along with size markers, for 16–20 hr at 20 V in 0.7% agarose gels in TBE buffer containing ethidium bromide at 0.5  $\mu$ g/ml. The DNA was transferred to nitrocellulose as described by Southern (12). The labeled oligonucleotides were hybridized to the DNA blots as described above for plaque filter hybridization.

RNA was fractionated in 0.75% agarose gels containing 2.2 M formaldehyde. Prior to sample loading, RNA samples were heated at 55°C for 15 min in Mops buffer [10 mM 3-(*N*-morpholino)propanesulfonic acid/5 mM Na acetate/1 mM EDTA, pH 7.0] containing 50% formamide and 2.2 M formaldehyde. Electrophoresis was run at 3 V/cm for 5 hr. The separated *Drosophila* RNA was transferred to nitrocellulose as described by Maniatis *et al.* (10). HindIII-digested  $\lambda$  DNA fragments served as molecular weight standards and were

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Abbreviations: MAb, monoclonal antibody; kb, kilobase(s).

visualized by ethidium bromide fluorescence.

For RNA blots,  $\lambda$ Dm24B10 DNA was labeled by nick-translation for 60 min at 15°C in a 50- $\mu$ l reaction mixture containing 0.5–1.0  $\mu$ g of DNA, 50 mM Tris Cl (pH 7.5), 8 mM MgCl<sub>2</sub>, 50 mM NaCl, heat-denatured bovine serum albumin (100  $\mu$ g/ml), 5 mM dithiothreitol, 20  $\mu$ M each dGTP, dCTP, and dATP, 0.8  $\mu$ M [<sup>32</sup>P]dTTP (3000 Ci/mmol), DNase I (80 ng/ml), and DNA polymerase I ( $3 \times 10^2$  units/ml). The filters were incubated with <sup>32</sup>P-labeled  $\lambda$ Dm24B10 ( $1.5 \times 10^8$  cpm/ $\mu$ g;  $7.6 \times 10^7$  cpm) for 32–40 hr at 68°C in 6 $\times$  NaCl/Cit/5 $\times$  Denhardt's solution/calf thymus DNA (100  $\mu$ g/ml)/0.5% NaDodSO<sub>4</sub>/10% dextran sulfate. The filters were washed for 5 min in 2 $\times$  NaCl/Cit/0.5% NaDodSO<sub>4</sub> at room temperature, for 15 min in 2 $\times$  NaCl/Cit/0.1% NaDodSO<sub>4</sub> at room temperature, and for 2 hr in 0.1 $\times$  NaCl/Cit/0.5% NaDodSO<sub>4</sub> at 68°C. The filters were exposed to XAR-5 film at –70°C for 2–14 days.

**DNA Sequencing.** Both strands of the *Hae* III/*Hinf* I fragment (Fig. 2) were sequenced using the Maxam and Gilbert technique (13). To do this, the fragments were asymmetricaly end-labeled by cutting the *Pst* I/*Eco*RI fragment separately with either *Hae* III or *Hinf* I. Each resulting digest was treated with bacterial alkaline phosphatase to remove unlabeled 5' phosphates and then end-labeled using [<sup>32</sup>P]ATP and T4 polynucleotide kinase, as described above for oligonucleotides. The labeled *Hae* III/*Pst* I and *Hinf* I/*Eco*RI fragments were then further cleaved with *Hinf* I and *Hae* III, respectively, giving rise to two *Hae* III/*Hinf* I fragments each with <sup>32</sup>P-label on a different strand. The labeled fragments were purified by gel electrophoresis and electroelution. The samples were treated with dimethylsulfate (G-specific), hydrazine (C and C+T), alkali (A>C), and formic acid (A+G). Nucleotides 45–148 were determined by twice-repeated sequencing of both strands; nucleotides 8–45 and 149–225 were determined by twice-repeated sequencing of single strands, and nucleotides 1–8 and 225–240 were determined only once, each on a single strand.

**In Situ Chromosomal Hybridization.** *In situ* hybridization to giant salivary gland chromosomes of *Drosophila* third instar larvae was done as described by Pardue and Gall (14), using the <sup>35</sup>S-labeled *Eco*RI/*Pst* I restriction fragment (Fig. 2) at a specific activity of 5–8  $\times 10^7$  cpm/ $\mu$ g. The hybridization step was done for 24 hr at room temperature, followed by washing for 10 hr in 0.36 M NaCl/0.02 M NaH<sub>2</sub>PO<sub>4</sub>, pH 7.4/2 mM EDTA at room temperature. The slides then were coated with Kodak NTB3 emulsion, exposed for 5–12 hr at 4°C, and developed in Kodak D11. Slides were fixed in acetic acid/ethanol (1:3, vol/vol) for 2–3 min and stained with 0.1% Giemsa stain in 10 mM Na phosphate buffer, pH 7.4.

## RESULTS

**Photoreceptor Cell Antigen 24B10.** MAb24B10 is produced by a hybridoma derived from spleens of mice immunized with retinal homogenates (4). Immunohistochemical staining of cryostat sections of adult tissue with this MAb is highly specific for photoreceptor cells and their axons (Fig. 1A) (4). During the third instar of larval development, staining is observed in the primordium of the adult eye (the eye imaginal disc), illuminating the developing photoreceptor cell bodies and their processes extending into the developing brain. The larva also contains a photosensitive organ near the pharyngeal complex (15), the cells and axons of which are stained with this MAb. The antigen recognized by MAb24B10 (Ag24B10) has been purified by immunoaffinity chromatography and shown to be a 160-kDa glycoprotein (Fig. 1B) comprising about 0.8% of the total eye protein (5). As a first step toward cloning the gene encoding the polypeptide chain, the sequence of 19 of the first 21 amino acids at its

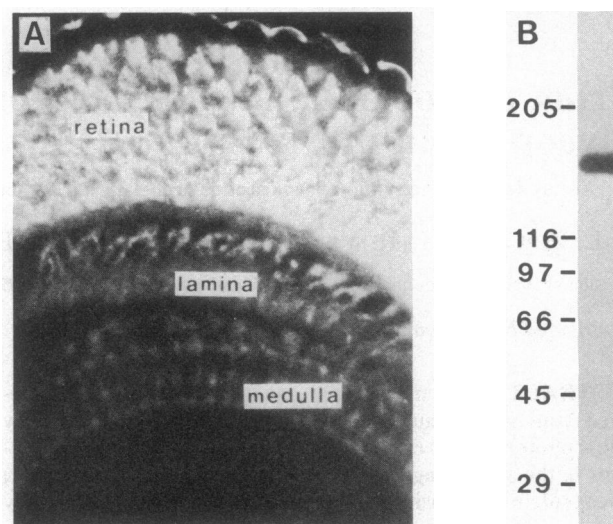


FIG. 1. (A) Photoreceptor cells and their axons in the *Drosophila* visual system stained by MAb24B10. A 2- $\mu$ m cryostat section was stained by indirect immunofluorescence. In the retina layer, photoreceptor cell clusters are seen. The projections of the axons from receptor cells 1–6 into the first optic ganglion (lamina) and from receptor cells 7 and 8 into the second optic ganglion (medulla) can also be seen. ( $\times 250$ .) (B) Electrophoretic pattern of the corresponding antigen, Ag24B10, a 160-kDa polypeptide, which was purified by immunoaffinity chromatography from a fly head homogenate, labeled with <sup>125</sup>I, and electrophoresed through a linear 5–15% gradient NaDodSO<sub>4</sub>/polyacrylamide gel and autoradiographed. (See ref. 5.) Markers at left are in kDa.

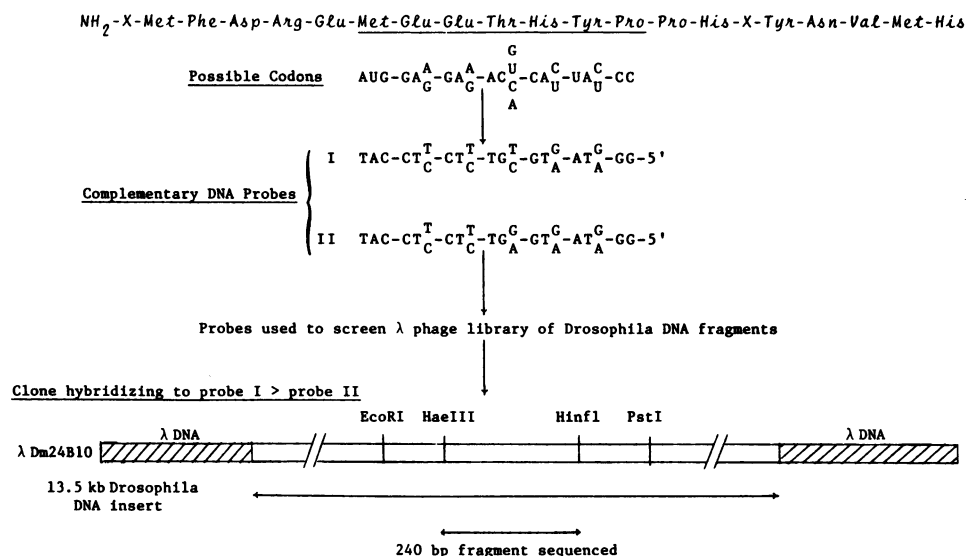
NH<sub>2</sub> terminus was determined (5). The sequence is shown in Fig. 2.

**Cloning the Gene for Ag24B10.** From the amino acid sequence and the genetic code, a probe for isolating the Ag24B10 gene was designed. A stretch of seven amino acids (Fig. 2) was selected for this purpose. Because of degeneracy in the genetic code, the nucleotide sequence in the *Drosophila* genome encoding this stretch could be one of 64 different possibilities, each 20 nucleotides in length. The third base position of the proline codon was omitted, since it would have multiplied the number of possibilities by another factor of four. The probability that a given one of these oligonucleotide sequences would occur at random in a genome the size of *Drosophila*'s is about  $3 \times 10^{-4}$ .<sup>\*</sup> The probability that any of the 64 could so occur is thus only about 2%. Therefore, we anticipated that these probes would be highly specific for the Ag24B10 gene.

In view of the four possible bases of the threonine codon, two separate probe populations were synthesized, one a mixture of 32 oligomers with purines at position 12, the other with pyrimidines at this position. The hybridization conditions used to screen the recombinant DNA library (see *Materials and Methods*) were designed so that the probe population containing an exact match would bind more strongly to the correct genomic sequence than would the other mixture, which would contain at best one mismatched base.

About 35,000 plaques of a *Drosophila* genomic DNA library in phage  $\lambda$  Charon 4 (7) were blotted to duplicate fil-

<sup>\*</sup>The probability that any of these sequences would occur at random in the *Drosophila* genome may be roughly estimated as follows. Any 20-base segment in the genome could have one of  $4^{20}$  ( $1.1 \times 10^{12}$ ) possible sequences. With the simplifying assumption of equal frequency and random order of the bases, the chance that a given 20-mer oligonucleotide would match a random 20-base segment of genomic DNA is then  $1/(1.1 \times 10^{12})$ . Since the *Drosophila* genome contains  $1.7 \times 10^8$  base pairs, a base on either strand being a possible starting point for a 20-base segment, the chance that a fortuitous match would occur is  $3.4 \times 10^8/(1.1 \times 10^{12}) = 3.3 \times 10^{-4}$ .

NH<sub>2</sub>-terminal Protein Sequence of Ag24B10

ters. One set was hybridized to probe I and the other to probe II. One plaque of the library was identified that hybridized to both probe populations, binding more strongly to probe I. This phage was designated λDm24B10. DNA from this clone and *Drosophila* genomic DNA were digested with restriction endonucleases *Eco*RI, *Hind*III, and *Bam*HI and compared on DNA blots probed with probe I. As shown in Fig. 3, a single band hybridized in each digest; the λDm24B10 and genomic DNA blots were indistinguishable. This showed that the *Drosophila* DNA insert λDm24B10 contains the only sequence of the genome that bound to the probe. Furthermore, the presence of a single hybridizing band in digests of genomic DNA is consistent with the possi-

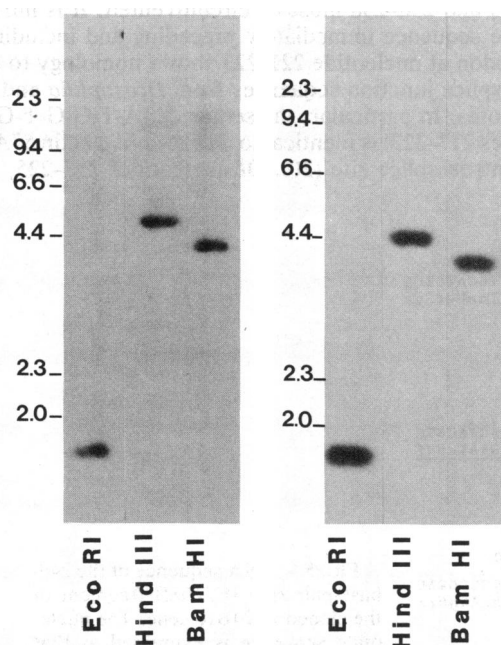


FIG. 3. DNA blots comparing clone λDm24B10 with genomic DNA. *Drosophila* genomic DNA (Left) and λDm24B10 cloned DNA (Right) were digested with restriction endonucleases *Eco*RI, *Hind*III, and *Bam*HI. After agarose gel electrophoresis and transfer to nitrocellulose, these digests were hybridized with <sup>32</sup>P-labeled oligonucleotide probe mixture I. Size markers (in kb) indicate positions of fragments from *Hind*III-digested λ DNA.

FIG. 2. Isolation of DNA clone encoding Ag24B10. The NH<sub>2</sub>-terminal amino acid sequence was determined with a protein microsequenator (5). X indicates a position at which no amino acid assignment was made. The underlined stretch of amino acids could be encoded by 64 different combinations of bases in mRNA. Two separate mixed probes were made: probe I with pyrimidines at position 12 and probe II with purines. The probes were 5' end-labeled and used to screen a λ Charon 4 *Drosophila* genomic DNA library. A clone was isolated that hybridized strongly to probe I and more weakly to probe II. A *Hin*PI/*Hae* III subfragment that bound the oligonucleotide was sequenced (Fig. 6). bp, Base pair.

bility that the gene occurs as a single copy per haploid genome.

**mRNA for the Ag24B10 Gene.** Total RNA, poly(A)<sup>-</sup> RNA, and poly(A)<sup>+</sup> RNA were isolated separately from adult heads and bodies. These were fractionated by agarose gel electrophoresis, transferred to nitrocellulose, and probed with <sup>32</sup>P-labeled DNA from λDm24B10. A polyadenylated transcript of 4.3 kb was observed in head RNA but not in body RNA (Fig. 4). This is consistent with the fact that heads but not bodies contain Ag24B10. This was shown by immunohistology of serial cryostat sections of entire adult flies. Also, in immunoadsorption experiments with MAb-24B10-beads the 160-kDa polypeptide was specifically adsorbed from head but not from body homogenates (unpub-

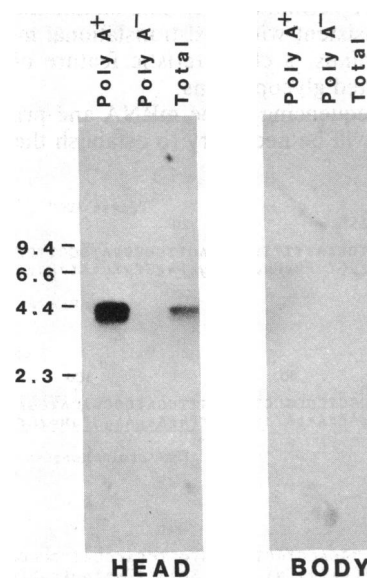


FIG. 4. RNA homologous to λDm24B10. Unfractionated RNA (10 μg), poly(A)<sup>-</sup> RNA (10 μg), and poly(A)<sup>+</sup> RNA (0.6 μg), prepared from fly heads or bodies, were electrophoresed on a 0.75% agarose/formaldehyde gel and then transferred to nitrocellulose. The blots were probed with <sup>32</sup>P-labeled λDm24B10. Molecular size markers were provided by a *Hind*III restriction digest of λ DNA. A single species of poly(A)<sup>+</sup> mRNA hybridized with the probe. It was present only in RNA from heads, consistent with the location of Ag24B10 specifically in the visual system.

lished data). An RNA of 4.3 kb could encode a polypeptide of 160 kDa. This coding capacity is consistent with the size of Ag24B10, as estimated by NaDodSO<sub>4</sub>/PAGE.

Levy and Manning (16), using the same phage  $\lambda$  genomic library as we did, isolated a series of DNA clones which bound poly(A)<sup>+</sup> RNA from *Drosophila* heads rather than bodies. By several criteria, one of their clones (designated 516) appears to be the same as  $\lambda$ Dm24B10. Both clones map to band 100 B (see below). The pattern of restriction fragments from an *EcoRI*/*HindIII* double digest appears the same (data not shown). On RNA blots, both clones recognize a high molecular weight head-specific poly(A)<sup>+</sup> messenger of  $\approx$ 4 kb. The abundance of the head-specific message bound by clone 516 is 0.12% of the head poly(A)<sup>+</sup> RNA. This is of the same order as the concentration of Ag24B10 (0.25% of total head protein), as determined by radioimmunoassay (5).

**Partial Sequence of the Cloned Gene.** To determine whether the  $\lambda$ Dm24B10 clone includes the coding sequence of the NH<sub>2</sub> terminus of Ag24B10, the region within which the oligonucleotide binds was sequenced. The  $\lambda$ Dm24B10 DNA was digested with a mixture of restriction enzymes *EcoRI* and *Pst* I. The DNA fragments then were separated by gel electrophoresis and a fragment 0.60 kb long, which DNA blot analysis showed to hybridize to probe I, was subcloned in the plasmid pUC8. Using a panel of restriction endonucleases, a smaller 240-base-pair *HinfI*/*Hae* III fragment was identified, which hybridized to probe I. This was sequenced by the procedure of Maxam and Gilbert (13) (Fig. 5).

The amino acid sequence corresponding to nucleotides 83–145 is in excellent agreement with the NH<sub>2</sub>-terminal amino acid sequence of Ag24B10 (5). From the DNA sequence, the amino acids at positions 15 and 21 are cysteines rather than histidines. Reexamination of the protein sequencing data showed that these residues were originally misidentified, due to inadequate resolution of >PhNCS-cysteine and >PhNCS-histidine in the HPLC system. The residue at the NH<sub>2</sub> terminus of Ag24B10 was not clearly identified in the protein microsequencing. The presence of an amino acid residue at the NH<sub>2</sub> terminus other than methionine, in this case serine, is consistent with post-translational modification of the NH<sub>2</sub> terminus, a characteristic feature of many membrane-associated glycoproteins.

Although sequencing of the mRNA and primary translation product will be necessary to establish the organization

of the gene, several features of the DNA sequence may be noted. The sequence upstream from the serine codon (nucleotides 83–85) is consistent with the possibility that it encodes the NH<sub>2</sub> terminus of a glycoprotein. An alanine preceding an NH<sub>2</sub>-terminal amino acid is a common site for cleavage by signal peptidases (17). Also, the amino acid residues translated from nucleotide 2 to the NH<sub>2</sub>-terminal serine are markedly hydrophobic (70% compared to 30% downstream), as expected for signal sequences. Protein synthesis is often initiated at the first AUG encountered from the 5' end of the mRNA (18). However, in this sequence translation from the first AUG (corresponding to ATG in the DNA at nucleotides 27–29), while beginning with methionine, would encounter four stop codons in the subsequent 10, as shown in Fig. 5. If protein synthesis is initiated at the first methionine from the *Hae* III end (nucleotides 50–52), in phase with the serine at nucleotide 83–85, the primary translation product will contain a short hydrophobic signal sequence of 11 residues, of which 9 are hydrophobic. However, a length of 11 amino acids is somewhat shorter than the typical length of 14–30 (17). Primer extension experiments, using probe mixture I to prime the synthesis of cDNA from head poly(A)<sup>+</sup> RNA, indicate that the mRNA start site is about 280 nucleotides upstream of the *Hae* III site at position 1 in Fig. 5 (data not shown). It is possible, therefore, that translation is initiated upstream of the *Hae* III cleavage site, giving rise to a longer signal sequence. Another possibility is that removal of an intron from the upstream sequence generates a different signal sequence, as has been observed for *Drosophila* cuticle genes (19).

The *Hae* III/*HinfI* fragment has an open reading frame from the *Hae* III end (nucleotide 2) to nucleotide 220, where it is terminated by a stop codon, followed by another five codons downstream. From the apparent molecular weight of Ag24B10, the polypeptide should contain some 1400 amino acid residues. Therefore, this DNA fragment accounts for only a fraction of the entire protein. For this sequence to encode a portion of Ag24B10, the stop codons at nucleotides 221–223 and 236–238 must be circumvented. It is intriguing that the sequence immediately preceding and including the stop codon at nucleotide 221–223 shows homology to exon/intron splice junction sequences from *Drosophila* and other eukaryotes. In particular, the sequence -A-T-G-G-T-G- (nucleotides 217–222) is identical to a *Drosophila* actin 57A gene exon/intron splice site (20). Of nucleotides 217–225, six of

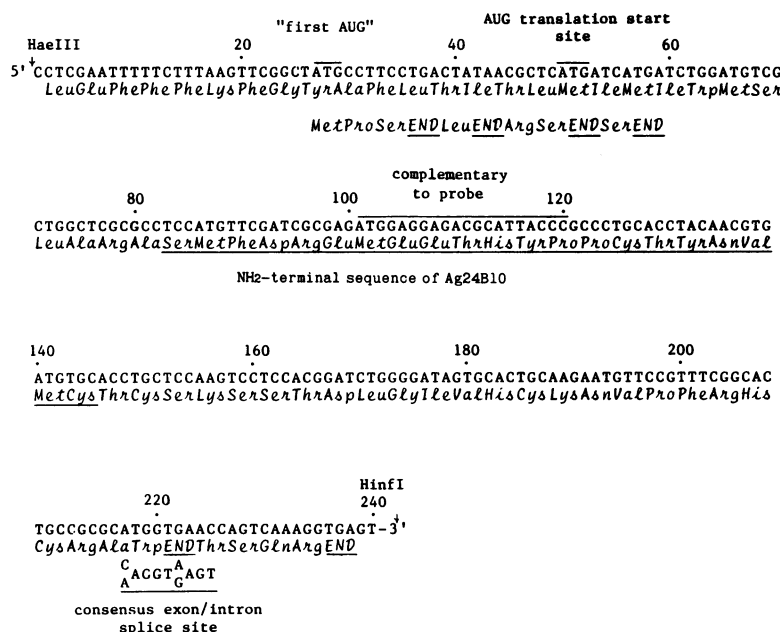


FIG. 5. DNA sequence of the 240-base-pair *Hae* III/*HinfI* fragment of the cloned Ag24B10 gene. The nucleotide sequence is expressed as that which corresponds to the mRNA. Directly beneath are shown the amino acid residues encoded by each triplet codon, in a reading frame in phase with the serine codon (nucleotides 101–103). This amino acid sequence is consistent with that determined for the NH<sub>2</sub>-terminal end of the protein.

nine are homologous to a consensus sequence (Fig. 5) determined from a survey of 139 splice sites in various organisms (21).

**Chromosomal Location of Ag24B10 Gene.** The *EcoRI/Pst I* fragment was <sup>35</sup>S-labeled and hybridized *in situ* to the giant salivary gland chromosomes of the third instar larva, followed by autoradiography and light microscopy (see *Materials and Methods*). A single hybridization site was observed at band 100 B near the distal end of the right arm of chromosome 3 (Fig. 6).

Two mutations that affect the visual system are also located near the tip of 3R. One mutant, transient receptor potential mutant (*trp*) (22), has a physiological defect in photoreception, but the *trp* mutation has recently been mapped to 99C (23) and is thus at least 25 bands away from the Ag24B10 gene. The second eye mutation in this region, loboid (*ld*), results in abnormal eye structure, but its exact position is not known (24).

## DISCUSSION

Immunological and molecular genetic techniques permit us to approach important questions concerning neuronal diversity. What polypeptides are differentially distributed among neurons? What functions do they subserve? How is this diversity generated; i.e., what regulates the expression of particular subsets of genes in a given neuron?

MAbs have been used to identify polypeptides unique to specific neuronal cell types in *Drosophila* and other organisms. We chose to study Ag24B10 because of its specificity to one neuronal type and its early appearance during eye development. Several lines of evidence indicate that the identified DNA segment encodes part of the 160-kDa photoreceptor-specific glycoprotein, Ag24B10. First, the DNA sequence and protein microsequencing data are in agreement. Second, the DNA sequence codes for a non-methionine (serine) NH<sub>2</sub>-terminal amino acid, a putative signal endopeptidase cleavage site, and an upstream hydrophobic sequence. All of these are characteristic of glycoprotein genes. Finally, a head-specific transcript of 4.3 kb is of the correct size to encode a head-specific glycoprotein of 160 kDa.

The paradigm illustrated here makes use of a MAb to identify a polypeptide that earmarks a given neuronal cell type and to isolate that molecule. Subsequent steps lead to the identification of a gene whose expression is characteristic of that cell type, and altering the gene provides a way to identify

the function of the molecule. In the *Drosophila* eye, the anatomy, physiology, and development of photoreceptor cells are known in considerable detail. This should aid in the analysis of mutant phenotypes. Detailed knowledge of the gene's structure should facilitate the study of the mechanisms by which the expression of this gene is restricted to photoreceptors.

The isolation of neuron-specific genes in *Drosophila* is a step toward the design of neuron-specific DNA vectors, potentially powerful reagents for studying neural development and function. Such vectors would permit the introduction and expression of genes not normally expressed in a given neuron. To develop these vectors, *cis*-acting regulatory sequences that confer specificity of expression to a given neuron must be identified. *P*-factor-mediated DNA transformation facilitates the identification of such sequences (25–27) and the subsequent introduction of hybrid genes into the *Drosophila* germ line. Thus, molecular genetic study of neuron-specific genes, such as that encoding Ag24B10, may lead to methods for selectively manipulating a particular class of neurons in an otherwise normal organism.

We thank Drs. Dennis Ballinger, Elliot Meyerowitz, and David Teplow for helpful discussion and, in particular, Dr. Meyerowitz for generously providing laboratory facilities. This work was supported by the Helen Hay Whitney Foundation (S.L.Z.), the Gosney Foundation (T.R.V.), and National Science Foundation Grant PCM 79-11771 (S.B.).

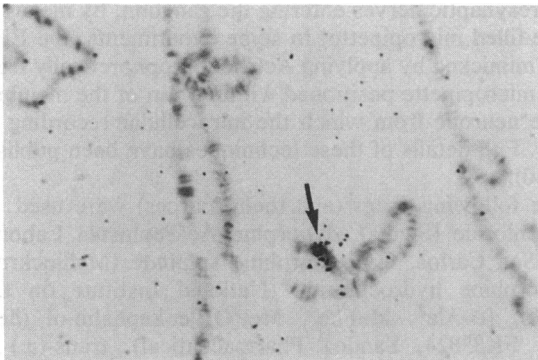


FIG. 6. Chromosomal localization of the Ag24B10 gene. An *EcoRI/Pst I* fragment of the  $\lambda$ Dm24B10 clone (Fig. 2) was labeled with 5'-[ $\alpha$ -<sup>35</sup>S]thio]dATP by nick-translation, hybridized to squashed preparations of larval salivary gland chromosomes, and autoradiographed. Arrow indicates site of hybridization at band 100 B, near the tip of the right arm of chromosome 3.

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